ABSTRACT

Acid snow of a pH range between 4.38 and 5.13 was observed in a watershed of Shokanbetsu River in northwestern Hokkaido in 2001-2003. The pH of the stream water decreased to 6.74 in the mainstream of Shokanbetsu River and 6.28 in the tributary during the spring melt runoff. The acidity of the snow is neutralized when snow melt water trickles from the ground surface and when flowing from the tributary to the mainstream. Although salmon juveniles regulated their serum Na⁺ levels in acid water of a pH 5, the serum Na⁺ levels decreased in the smoltification season. The acidity of the stream water in Shokanbetsu River was not lethal to salmonids in Hokkaido.

KEY WORDS: Snow melt water; pH; Hokkaido; Salmon; Serum Na⁺

INTRODUCTION

Hokkaido, the northern island of Japan, is covered with ice and snow in winter. Four species of Pacific Salmon *Onchorhynchus*, two species of Charr *Salvelinus*, and Japanese huchen *Hucho perryi* are naturally distributed over Hokkaido. Most of the salmon species in Hokkaido undergo external and internal transformations to preadapt for the marine environment. Both chum salmon *Onchorhynchus keta* and pink salmon *O. gorbusha*, a major fish for the commercial fisheries in Hokkaido, migrate downstream to the ocean in April soon after emergence. Masu salmon *O. masou* is only distributed in East Asia including Japan. Most masu salmon in Hokkaido migrate down to the ocean during spring-early summer one or more years after living in streams. The land-locked sockeye salmon *O. nerka* have been transplanted from Lake Akan in Hokkaido to several lakes in northern Japan. The under-yearling juveniles migrate to a lake during spring-early summer. Japanese charr *Salvelinus leucomaenis* is distributed in the main island of Japan and Hokkaido. The yearling charr in Hokkaido migrate to the ocean in spring after the parr-smolt transformation. Land-locked Dolly varden charr *S. malma* and Japanese huchen are distributed only in Hokkaido. Japanese huchen spawn in spring-early summer, although other salmonids in Japan
spawn in fall.

In Europe and eastern North America, freshwater ecosystems have been damaged by acidification due to acid snow and acid rain (Steinberg and Wright, 1994). In spring, the rapid inflows of acid pollutants into rivers have destroyed salmon populations in Europe and North America (e.g., Leivestad and Muniz, 1976). On the other hand, the industrial processes in East Asia discharge NOx and SOx into the air. These acid pollutants are transported to the coast of the Japan Sea in Japan by westerly wind during winter (Japan Environmental Agency, 1997). The snow in the coast of the Japan Sea in Hokkaido accumulated high concentrations of H\(^+\) and SO\(_4\)\(^{2-}\) (Noguchi et al., 2001). Acid impacts on the salmonid populations are also a concern in Hokkaido. Shokanbetsu River is located in the watershed of the Mashike Mountains region along the Japan Sea in northwestern Hokkaido, and has heavy snowfall. It is possible to consider that the Shokanbetsu River System is greatly influenced by the acid pollutants from East Asia in winter. We investigated the snow chemistry and the chemistry of stream water in the Shokanbetsu River region during 2001-2003. Then, environmental influence of the acid water on salmonid fish was examined, based on osmoregulation during the smoltification season, to evaluate the acid impact on the commercially important salmon fisheries in Hokkaido.

**STUDY AREA**

The study was conducted in the Shokanbetsu River System, northwestern Hokkaido, in 2001-2003 (Figure 1). The whole of the watershed areas and the length of the mainstream of Shokanbetsu River and of the tributary Nenosawa River are 99.2 km\(^2\) and 26 km and 2.5 km\(^2\) and 2.6 km, respectively. Snow at a sampling station near the Douhoku Branch of Hokkaido Fish Hatchery by Shokanbetsu River was collected to measure the pH and electric
conductivity after thawing at room temperature, and snow depth was measured (Figure 1). Water samples were collected at the mainstream, tributary Nenosawa River, and a small branch of Nenosawa River from February 2001 to July 2003 (Figure 1). The stream water samples in the main stream were sampled at a point 7 km up from the river mouth. Nenosawa River flows into Shokanbetsu River at a point 6 km up from the river mouth.

MATERIALS AND METHODS

Snow samples were collected by cutting blocks of snow measuring 30-50 cm on 25 February 2001, 15 February 2002, and 21 March 2003. The snow samples were transferred to the laboratory and thawed at room temperature to measure the pH and electric conductivity (EC) using a digital pH meter (D-21, Horiba) and an EC meter (CM-14P, Toa). Water samples were collected every day in spring, and also collected monthly in the other seasons. As soon as the atmospheric and water temperature, and the pH and EC of each sample was measured, pH 4.8 alkalinity was determined by titration with 0.02 N H2SO4 at room temperature. The NO3⁻ in the water samples in 2001 was measured by FIA (5012, Tecator) cupper/cadmium reduction method and the SO4²⁻ was measured by ion chromatography (CCP&8020, Tosoh).

Chum salmon fry, underyearlings (0⁺) of sockeye salmon, and yearlings (1⁺) of masu salmon were exposed to acid water at pH 5 and control fresh water for 24 h during the migratory period (chum in April, other species in June) and in the post migratory period (August; no chum after the migratory season). The fish (n=10 with a tank) were transferred from a fresh water stock tank to a 20 L acid water tank (130 L for masu). The acidity was regulated by a pH controller (NpH680D, Nissin Rika) with a perista pump titrating 1 N sulfuric acid. The water temperature range was 8-10 °C (April), 10-12 °C (June), and 10-15 °C (August). Chum salmon fry and yearlings (1⁺) of masu salmon were exposed to snow melt water (200 L tank) at pH 4.89-4.95 and stream water (200 L) at pH 6.84-6.95 of Shokanbetsu River for 24 h in April 2002 (n=10 with a tank). The snow was transported from the watershed in Shokanbetsu River. The water temperature was 9.1-10.6 °C. These data are from Watanabe et al. (1995) and Watanabe et al. (2004). The blood was collected 24 h after acid water or snow melt water and control fresh water exposure. The blood was centrifuged at 10,000 rpm for 5 min and the serum samples frozen at −80 °C. Serum Na⁺ concentrations were measured by an atomic absorption spectrophotometer (180-50 and Z-6000, Hitachi). An analysis of variance (ANOVA) and Duncan’s new multiple range test was performed for statistical analysis.

RESULTS AND DISCUSSION

Depression of pH in the Shokanbetsu River System in spring
The pH and EC of snow in the watershed of Shokanbetsu River in 2001-2003 are shown in Table 1. The pH of snow was in the range of 4.38-5.13 in 2001-2003. The concentrations of H⁺ and non-sea salt (nss-) SO₄²⁻ in snow were observed higher in the coastal region of Japan Sea than in other regions in Hokkaido (Noguchi et al., 2001). These results suggest that the high concentrations of H⁺ and nss-SO₄²⁻ carried by westerly winds cause the lower pH of snow in the watershed of the Shokanbetsu River System.

Slight decreases of the water pH were observed continually in a small branch of a tributary of Shokanbetsu River during the spring melt runoff of 2001-2003 (Figure 2). The pH values were 6.74 in the mainstream and 6.28 in the small branch of the tributary in the season. The pH of stream water was higher than that of snow. The differences in the pH values between the snow and the stream water are in the range of 1−2 in pH, implying that large quantities of H⁺, SO₄²⁻, NOx and other ions were neutralized before the melt water reached the stream. Similarly, a slight acidification in the melt water in the stream was observed in the Okusawa Reservoir region of Otaru in the western Hokkaido facing to the Japan Sea (Aga et al., 2001). The observation and our present study indicate that the stream water in the western Hokkaido may be slightly acidifying during the spring melt runoff. At a riverhead of the Agano River in Fukushima Prefecture, the northern main island of Japan, the melt water is approximately pH 5.6 (Suzuki, 2003). In the present study, the EC peaked over 10 mSm⁻¹ in March at the early snowmelt in 2001-2003. The NO₃⁻ and SO₄²⁻ ions also peaked in March 2001 (Watanabe et al., 2004). In Norwegian river, pH shock is defined as occurring when the pH of a stream reaches a certain minimum due to the release of H⁺ and SO₄²⁻ during the early snowmelt (Johannessen et al., 1980). These studies suggest that the fluvial fishes, especially smolting salmonids in our study area, are exposed to pH shock in the thaw season even though it may not be as serious as in the case of Norwegian rivers.

### Table 1. Electric conductivities and pH values of snow melt water at different layers of snow columns collected at a bank of Shokanbetsu River, 2001-2003.

<table>
<thead>
<tr>
<th>Date</th>
<th>Height from bottom (cm)</th>
<th>pH</th>
<th>EC (mSm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 Feb.</td>
<td>253-200</td>
<td>4.95</td>
<td>4.72</td>
</tr>
<tr>
<td>2001</td>
<td>200-150</td>
<td>4.63</td>
<td>8.38</td>
</tr>
<tr>
<td></td>
<td>150-100</td>
<td>4.59</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td>100-50</td>
<td>4.61</td>
<td>14.22</td>
</tr>
<tr>
<td></td>
<td>50-0</td>
<td>4.60</td>
<td>6.08</td>
</tr>
<tr>
<td>15 Feb.</td>
<td>184-150</td>
<td>4.55</td>
<td>3.39</td>
</tr>
<tr>
<td>2002</td>
<td>150-110</td>
<td>4.44</td>
<td>3.73</td>
</tr>
<tr>
<td></td>
<td>110-77</td>
<td>4.85</td>
<td>2.99</td>
</tr>
<tr>
<td></td>
<td>77-50</td>
<td>4.38</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td>50-25</td>
<td>4.46</td>
<td>6.02</td>
</tr>
<tr>
<td></td>
<td>25-0</td>
<td>5.02</td>
<td>3.41</td>
</tr>
<tr>
<td>21 Mar.</td>
<td>230-195</td>
<td>4.75</td>
<td>5.01</td>
</tr>
<tr>
<td>2003</td>
<td>195-140</td>
<td>4.61</td>
<td>6.67</td>
</tr>
<tr>
<td></td>
<td>14-90</td>
<td>4.61</td>
<td>5.47</td>
</tr>
<tr>
<td></td>
<td>90-45</td>
<td>4.73</td>
<td>4.97</td>
</tr>
<tr>
<td></td>
<td>45-0</td>
<td>5.13</td>
<td>3.41</td>
</tr>
</tbody>
</table>

**Osmoregulation of Japanese Salmonids exposed to acid water**

In fish gills, the H⁺ ion in acidic water causes the loss of Ca²⁺ from gill epithelium, thus it induces reduction in the ability to control ion permeability, thereby causing ion regulatory disturbance and lose of Na⁺ and Cl⁻ (Rosseland and Staurnes, 1994). Yearlings of land-locked Japanese charr S.leucomaenis lost the ability to regulate the osmolality, Na⁺, and Cl⁻ ions in plasma in the sulfuric acid water at pH3.9 for 2-5 days; however, the charr maintained the...
osmoregulation in the fresh water of pH 4.6 for 1 week (Iwata et al., 1990). Although another land-locked species of rainbow trout *O. mykiss* showed decreased the blood Na⁺ concentration 3 days after exposure to acid water at pH 5.5, and then the blood Na⁺ concentration recovered after 7 days (Yada, 2000).

Figure 2. Seasonal changes in atmospheric and water temperatures (upper) and water pH (below) in the Shokanbetsu River System from February 2001 to July 2003. Inserted left-upper panels show snow depth measured at the Douhoku Branch of the Hokkaido Fish Hatchery.
The three species of anadromous salmon in the present study were exposed to acid water of pH 5 for 1 day, and then the serum Na⁺ concentrations of chum salmon *Oncorhynchus keta* fry, masu salmon *O. masou* smolts, and sockeye salmon *O. nerka* smolts decreased in their migratory seasons (Figure 3). In the masu and sockeye salmon after their migratory seasons, however, the serum Na⁺ concentrations did not change in the acid water. We also examined the changes in serum Na⁺ concentrations of the chum salmon fry and the pre-smolt masu in the snow melt water of pH 4.89-4.95 (Figure 4). The serum Na⁺ concentration of chum fry decreased, whereas the masu pre-smolts did not show the change. The chum fry with a small quantity of yolk remaining acquires seawater adaptability (Iwata *et al.*, 1982), and also they

Figure 3. Serum Na⁺ concentrations of pacific salmons exposed to pH5 acid water for 24 h in their seaward migratory and the post migratory season. *Significantly different at *P*<0.05 when compared with the concentration of the FW control.

Figure 4. Serum Na⁺ concentrations of 0⁺ chum fry and 1⁺ masu salmon juveniles exposed to snow melt water of pH 4.89-4.95 and pH 6.84-6.95 of stream water for 24 h in April. *Significantly different at *P*<0.05 when compared with the concentration of the FW control.
are obligatory sea-run migrants, the same as pink salmon, despite masu salmon having to complete parr-smolt transformation for their ocean life. The hyper-osmoregulatory ability of masu salmon is maintained in the pre-smolt and post-smolt stages except for the period in the fully smoltified stage. These differences in obtaining osmoregulatory ability may be reflected to the difference in their serum ion regulations. These results indicate that although non-migratory salmonids such as Japanese char and rainbow trout show the same resistance to the acid water as masu juveniles at not full-smolt stages, migratory salmon such as chum salmon and sockeye salmon and full smolt masu salmon are not resistant to acid water, because of obtaining their hypo-osmoregulatory ability.

Influence of snow melt water on salmonids

We observed the pH 6.28 of the stream water in the Nenosawa tributary and pH 6.74 in the mainstream of Shokanbetsu River at 6 or 7 km from the mouth of the river, respectively. The acid water at pH 5 decreases the blood Na⁺ concentrations of the chum fry, sockeye smolts, and masu salmon smolts in their migratory period (Figure 3, 4). Although the stream water at pH 6.28 is not lethal to the juvenile salmon, chronic exposure to the acidic thaw water may disable the smoltification processes and the downstream behavior of migrants when the fish encounter the spring thaw. Acid water of less than pH 6.4 inhibits spawning related nest-digging behavior of female sockeye salmon (Kitamura and Ikuta, 2000). The spawning behavior of Japanese char is also inhibited by acid water of less than pH 5.8 (Ikuta et al., 2003). Japanese huchen spawn from spring to early summer; the acid thaw may also influence their spawning.

CONCLUSIONS

Acid snow at pH 4.38-5.13 and stream water at pH 6.28 in the tributary were observed in the Shokanbetsu River regions. Acid water at pH 5 decreases the blood Na⁺ concentrations of chum fry, sockeye smolts, and masu salmon smolts in their migratory period. The pH of the stream water at pH 6.28 is not lethal to the juvenile salmon in western Hokkaido. Also, stream water at pH 6.28 may influence the spawning of Japanese huchen in Hokkaido.

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